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THE DEEP MYCOSES IN THEIR SURGICAL ASPECTS—THE RÔLE OF LABORATORY DIAGNOSIS*

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BACK of every assemblage, be it lay, or medical like this, of course there is a reason. The question can always be raised under such circumstances, "Why are we here?" To come immediately to the point—it is *fungus diseases* that you have chosen as a subject to engage such an outstanding institution as the California state meeting, and after that I dare say that it is the presence of a distant traveler like myself here that is the most cogent witness of the increasing importance of studies in general mycology and in your coccidioidal problem in particular. It is eloquent that you have seen fit to draft this willing conscript, involving over six thousand miles of time, thought and all that go with them.

This symposium, too, is something of a commentary—a compliment, on the level of medicine as practiced in California. By this I mean that, by and large, the type of subject engaging medical or other thoughts generally has its strongest appeal in connection with the bread and butter of everyday life; that is, for us, syphilis, tuberculosis, cancer, acute infectious fevers, and so forth. It is only after we feel that we have become more or less thoroughly acquainted (or perhaps better—self-satisfied) with these outstanding problems that we feel justified in undertaking adventures into less traveled fields in which the sights are at least new, even though they be less frequently met. Thus, to be concrete, the general practitioner, even if not a qualified expert, knows his pulmonary tuberculosis—after a number of years can arrive at a reasonably sure diagnosis. But now, while he still constitutes a real service to mankind at large, in the ordinary course of events he will encounter knotty and unusual cases from time to time where he will come to a standstill as to the final diagnosis; indeed, such cases are the ones that will be lasting memories with him—upstanding and permanent hurdles which he will view in retrospect and which will comprise the fascinating (or disgusting) problems to

vary the monotony of an otherwise colorless experience. Unless this practitioner is acquainted with the existence of such other mimics of tuberculosis, as syphilis, bronchiectasis, pulmonary spirochetosis, yeast infections, coccidioidal granuloma, aspergillosis, and so forth, he will not alone rob himself of the variety which leavens the routine, but he will not be doing his utmost for his fellow man.

To come at last to the point I am leading to, I take it that this meeting and this subject connote advance in California medicine consistent with the advances California has made in horticulture, agriculture, mining, engineering, shipping, the motion picture industry and the other members of the long, long list. That is, I would not claim that fungi are becoming increasingly important as pathogens; the probability is that they have been of comparable importance for centuries past; it is not that they are themselves rare, but that their recognition is rare. Now that they are becoming recognized—now that they have elbowed themselves so relentlessly into your medical picture, we have the spectacle here of Californians addressing themselves to the new problem as they have so successfully to tularemia, bubonic plague, botulism, foot and mouth disease, rabies, and a host of other diseases.

So here we are gathered; I daresay in the largest assemblage of mycological workers the United States has ever known—Ophüls, Rixford, Morrow, Montgomery, Moffitt, Meyer, Bowman, Cummins, to mention only a part. We are all members of a convocation for devising ways and means against a problem which fortunately (unlike cancer) has become concrete and offers a definite target at which to aim. My own humble part is to give you what I have on the basis of experiences which have been spread, if anything, more into the academic than the practical aspects of mycology.

SCOPE AND LIMITATIONS

I am glad indeed that I have been relieved of the necessity of including the superficial mycoses. Even confined as I am today to the deep ones, it is still a very large order, and you may well imagine that it will be necessary, and doubtless agreeable to you, that I but give you a general panorama of the situation. But at the same time, I will take the liberty of dipping into details from place to place where it becomes necessary to clear up certain misunderstandings that are particularly

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rife; which have baffled me in the past, and may save you some trouble. From conversations I have had, I take it that the average general pathologist of today is in very much the same situation mycologically that I was when I finished a ten years' training in general and special pathology under such masters as Allen J. Smith, Joseph McFarland, and Herbert Fox. With this realization I shall feel much less embarrassment in dwelling upon certain fundamentals.

As suggested, I shall confine my remarks to the following fungus diseases: streptothricosis (including actinomycosis), blastomycosis, torula infection, coccidioides infection, sporotrichosis, and miscellaneous ones such as Madura foot and "gummatous ulcers"; whereas, as tintured in the title, this presentation has two phases, one clinical and the other laboratory, I trust that they will be found to overlap just as all of our specialties overlap in best modern practice.

THE SURGICAL PHASE

In order to bring the matter home to you as forcibly as possible, I am going to ask the surgeon that he imagine, just for a moment, that there were no such institutions as the microscope, the test tube, the laboratory, and pathologist in general. Keep before your mind the list of deep mycoses already enumerated; that is, the streptothricoses including actinomycosis, blastomycosis, torula, sporotrichosis, and coccidioides. With syphilis and tuberculosis rampant as they are, what proportion of these mycoses do you imagine the average surgeon could pick up diagnostically? It goes without saying that the clear-cut cases of actinomycosis and Madura foot with their sulphur or other grains, the classical cases of sporotrichosis with the linear distribution of lesions along the course of lymphatics—a certain proportion of such could be separated from the general mélange of other indolent, ulcerative, sinus-producing, more or less proliferative, slowly destructive lesions of the skin, bone, and viscera. As to blastomycosis, most cases require dermatologic knowledge for diagnosis, but even here, as is even more the case with sporotrichosis, coccidioidal granuloma and the rest of the list, laboratory aides are becoming more and more requisite as we come to realize how varied the expressions of these deep mycoses may be and as we aim to approach 100 per cent efficiency instead of remaining satisfied with a respectable batting average. We have always invoked syphilis as the type example of a protean disease, but dermatologists by now know that tuberculosis is almost equally protean, and the California profession, I am sure, realizes that the mycoses likewise belong in the same list. Incidentally, the dermatologist is particularly appreciated when he relieves the situation with a diagnosis of bromid or iodid granuloma, or perhaps factitial dermatitis; all of these are conditions where he plays a unique part diagnostically.

I feel, therefore, that from the standpoint of surgical diagnosis, there are rather severe limita-

tions to the purely clinical approach. Only one or two of the mycoses named are clinically diagnosable 100 per cent under the best of conditions, and in the second place numerous cases of each will be so atypically expressed as to quite prevent the purely clinical diagnosis. Even the presence of syphilis and tuberculosis will not be final differential diagnostic criteria; with both diseases ubiquitous, it is quite obvious that mycosis may appear in a syphilitic or tuberculous subject equally as well as typhoid fever; this is all the more true in localities where fungus disease is endemic and where the factor of statistics thus does not play so large a differential part. Indeed, I feel that *the association of tuberculosis with mycosis should be tested with every case of proved mycosis*; certainly it is a principle that fungi become invasive much more readily in connection with preëxisting diseases. We have found this to be the case in connection with pulmonary aspergillosis of birds at our Philadelphia Zoölogical Garden^{1,2}; this point should be further inquired into as a generality for human mycotic disease.

The Therapeutic Phase.—The moment one mentions therapeutics in fungus diseases, the medical mind automatically turns to iodine. If there is such a thing as a list of specific remedies in medicine, iodine belongs on it in connection with the mycoses in general. Not that it is invariably a specific for a given mycosis, or that it is a specific for every mycotic disease, but as much can be credited to it as a specific as to mercury and arsenic in syphilis, iron in the anemias, quinine in malaria, and so forth. In none of the instances cited is specificity invariable and absolute. With iodine so useful, hence it is that, again, as in syphilis, essentially surgical procedures have limitations in connection with mycotic disease in general. By and large, in blastomycosis, sporotrichosis and some of the streptothricoses, the scalpel (certainly in the earlier stages of the disease) has no place until iodine has been tried. How well I recall that, as a resident, I assisted my chief to excise a "sarcoma" of the thigh; only when the clean wound declined to heal, and iodides were successfully resorted to, were we disillusioned. The patient had syphilis.

Streptothricoses.—To be a little more specific, I should say that in the deep streptothricoses, including actinomycosis, surgical treatment has in general proved unsatisfactory. Exceptions, it is true, there are. Iglick of Orland, California, has reported such success with you, but it was in a scalp case; that is, superficial as the surgeon views it. The experiences of Ophüls, Woolsey, Pritchard, Black, Rinkenberger, Caglieri, Frick, Loiseaux, all Californians, find place in Sanford's and Voelker's³ exhaustive compilation on actinomycosis. Of these, Caglieri's patient was operated upon and died, one of Black's refused operation and recovered (the follow-up notes are not supplied of his second case), and Iglick's patient was cured with potassium iodide. True, Iglick's was

a scalp case; and Ruhrah notes that cutaneous forms are decidedly hopeful as to prognosis. Ophüls' five cases had only a postmortem significance, but were valuable in indicating one of the factors concerned in inducing pulmonary gangrene. His organisms were not the conventional ray fungi but their first cousins, the streptothrices.

On the other hand, dermatologic experience is replete with examples of success where large doses of iodid (upward of 480 grains in twenty-four hours) has been accompanied by radiation. It appears that the combination of these two agents is necessary for consistent and optimum results. In streptothricosis (that is, where the organism concerned belongs to the threads, like actinomyces, but in which the ray arrangement does not take place) the same considerations obtain. In these latter we have, however, a much smaller group of materials from which to make deductions; the technical mycological study of these microorganisms has not been undertaken as fully as it should. Groupings cannot be made, and therefore no generalizations.

Incidentally, the concretions of the lachrymal duct which are commonly indexed as due to actinomyces, are not due to a ray fungus but simply to tangled threads of a streptothrix (*Actinomyces foersteri*).⁴ The organism is not a true pathogen, but produces disease only by its mechanical effects. The calcification is a secondary development.

Blastomycosis.—This is not essentially a surgical disease therapeutically. In the cases which have escaped recognition for many years and where extensive destruction of tissue has taken place, of course surgical corrections are indicated. In the early stages, however, iodids have the outstanding preference. The only exception to this rule that I know is a unique case where the organism assumed unseemly acute infective and invasive propensities and rapidly metastasized from the hand along the lymphatics into the axillary nodes. In this case the prompt and complete excision of the primary focus was performed solely for the purpose of eliminating the major reservoir of dissemination in the case. Intensive conjoined iodid and x-ray treatment resulted in a complete and apparently permanent cure.

Deep Torula Infection.—From the practical standpoint, this at present concerns mostly the meninges and brain. Here, obviously, iodine medication and x-ray are indicated, inasmuch as the disease is a widespread, diffuse one, and so located as to be impossible of surgical eradication.

Yeast infection of bone extends back to Busse's⁵ original classic of 1895. Since then, Brewer and Wood,⁶ as well as Connor,⁷ have reported cases in the American literature. I have had personal experience with cultures from pus from a case of osteomyelitis in the tibia of a child (Buzby's case).⁸ Channel operation and iodides resulted in complete cure.

In the genito-urinary tract we know of but few reports of yeast infection. This is rather surpris-

ing in view of the predilection which yeast infection has for the kidneys of experimental animals. Perhaps they are overlooked.

Sporotrichosis.—As in the case of blastomycosis, this should concern the surgeon operatively only when the disease has progressed far, has been neglected, and destroyed tissue to the extent that trimming up becomes necessary. Generalized cases of this disease are of course widely known. Even in those cases where operative procedures are in order, it goes without saying that iodids should be conjoined.

In the eye, sporotrichosis should not be forgotten; a localization over the lachrymal duct should provoke thought toward sporotrichosis. The Giffords⁹ of Omaha have done us real service in this connection. It is obvious what a boon iodid therapy is in a situation like this.

Coccidioidal Granuloma.—This is your field, and I cannot bring anything to you in re surgery. From what I have heard, the newer copper and antimonial therapy is only promising in the early cases or in cases like Guy and Jacobs,¹⁰ where the organism is of attenuated virulence. After that I take it that the operative treatment is of the same order as that for tuberculosis.

Under the "miscellaneous" heading I shall have nothing to say about the surgical aspects because the cases are quite too scattering etiologically to justify general remarks. In general, the diseases under this heading have the general characters of tuberculosis or syphilis, with gummas or gummatous ulcers picturing their low, indolent course, but caused by a wide variety of fungi (hormodendrum, mucor, cephalosporium, and so forth). They will be discussed in greater detail in the section on laboratory diagnosis.

Summary.—As the problem of the surgeon mycosis introduces itself first in *differentially diagnosing* certain subacute gummatous and ulcerative states which are analogous to tuberculosis and syphilis. By and large, this problem is more calculated to be solved by the laboratory man than by the Simon-pure clinician. Even where the diagnosis clinically appears quite clear it is generally advisable to confirm it by the laboratory test; even sporotrichosis may be mimicked by tuberculous lymphangitis of the extremities. After diagnosis the problem of *therapeutics* presents, and here again those procedures which are accredited as surgical are only applicable in selected instances. The experience of the dermatologist in the technique of intensive (intravenous) iodid and iodine medication, the application of x-rays, the administration of colloidal copper, antimony, and so forth, should be taken advantage of. After that there will still remain numerous instances where the life-saving skill, or at least the life-prolonging skill, of the surgeon must be gladly utilized.

LABORATORY DIAGNOSIS

The concern of the surgeon in the diagnosis of the mycoses will not stop at this point. Not

that he would, should, or could, busy as he is with his own specialty, master all angles of a complicated subject like mycology. He would do well, however, to have a general perspective of the scope and limitations of the laboratory diagnosis of the mycoses. Thus, I have already, in another place (11), indicated that pathologists, by and large, concede that they are woefully remiss in their mycological training. At once I would hurry to point out that this is no fault of theirs, but in the final analysis is due to the comparative infrequency with which calls for mycological examinations are made. That is, in routine laboratory work the bacteriologic and histologic aspects, and so forth, far overshadow the mycologic ones. It thus becomes physically impossible for the average pathologist to be expert also in the rarely trodden mycologic field. In California you are much better off than in other parts of the United States because the mycoses bulk much larger in your problems and your pathologists have learned to adapt themselves to at least certain needs; their average for mycologic efficiency rates much higher, I am sure, than elsewhere in the United States. As I see it, the solution lies not in requiring every pathologist to become proficient in all of the minutiae of mycological biology and diagnosis, but to create at least a few mycological centers geographically placed throughout the United States; an expert mycologist being located in each and having facilities to check up on the problems which are too much for the general pathologist at large.

After this the surgeon will readily see that he should have his ear constantly to the ground as to the mycologic qualifications of an otherwise perfectly efficient pathologist.

And then there is the contre coup. What is just as important, he may be glad to save himself some chagrin in respect to the tissue samples which he forwards (or does not forward) to the pathologist. Let me first beg for more and yet more *biopsies*. The procedure is so simple, so safe, and in many cases so illuminating and unequivocal that it ought never to be omitted in a section like California as long as the slightest doubt remains as to the diagnosis of a gumma-like ulcer. Even though the Wassermann reaction be positive, and even though the tubercle bacillus be demonstrated in smears, I feel that it is still incumbent, if not more than as a measure of California patriotism, to at the very least inquire for coccidioidal granuloma. You must determine whether these diseases are associated and whether it is this which is confusing you. The situation is much simpler than with the histologic diagnosis of/between tuberculosis and syphilis; you are fortunate in that coccidioides, in the present state of our knowledge, is invariably to be found if present, and is so large that it can scarcely be overlooked.

Likewise let me beg of you to request a larger number of *autopsies*. No doubt you are surfeited

by now with this appeal—it has become tiresome, but I would call your attention to an additional consideration in California that does not obtain elsewhere, that is, the magnitude and the menace of your coccidioidal problem; apart from the humanitarian aspect, what it means to your public health and this in turn to your economics. Is it the Mediterranean fruit-fly of your public health? I need but mention man-power, industry, labor problems, state appropriations for hospitals, sanatoria, and poorhouses to conjure up for you the ramifications of an endemic disease of the gravity of coccidioidal granuloma. You have made a worthy step in already making the disease reportable, and now, by correlating the morbid anatomical findings with the different systems of treatment employed; by detecting different grades of virulence in the different strains of microorganisms as shown by effects upon the viscera; by checking up on the question of an associated tuberculosis, syphilis, etc.—only by this order of attention and effort can the necessary funds of information be secured which in the aggregate will enable you to approach your problems on a secure foundation and after that with 100 per cent efficiency. Furthermore, as every investigator knows, some of the most worthwhile findings are those which develop only incidentally, as side lines in connection with work which was planned in quite a different direction. No one knows, when embarking upon an autopsy program, just what findings are quite unexpectedly going to be thrust upon him. Play the game with the pathologist; do not let him go it alone!

MYCOLOGIC TECHNIQUE

To the pathologist directly I cannot deliver a message except as conditioned, that is, expeditiously. Therefore I am confining myself to a table of amplified aphorisms, if you will, in re technique. This has been arranged on the basis that he is better acquainted with bacteriological technique than I am and that therefore I can place my materials before him most efficiently by beginning where bacteriology leaves off and thereafter indicating certain respects in which mycological technique differs from the bacteriological.

Use of NaOH in Direct Preparations.—NaOH (10 to 30 per cent) induces a difference in refractive index between fungus substance and the surrounding medium; this, in conjunction with the comparative grossness of fungus structures, has a use in mycology which it does not have in bacteriology. Except when extremely delicate fungi are to be expected like the streptothrices, it should never be omitted in immediate direct examination "in the wet" of pus, scrapings, tissue—in fact, any samples of material. The cutaneous fungi—blastomyces, coccidioides—indeed even the fine threads of *Microsporon minutissimum* may be readily identified thus. As far as I know, the sporotrichums resemble the nuclear fragments of leukocytes to such an extent that the diagnosis of sporotrichosis in direct prepa-

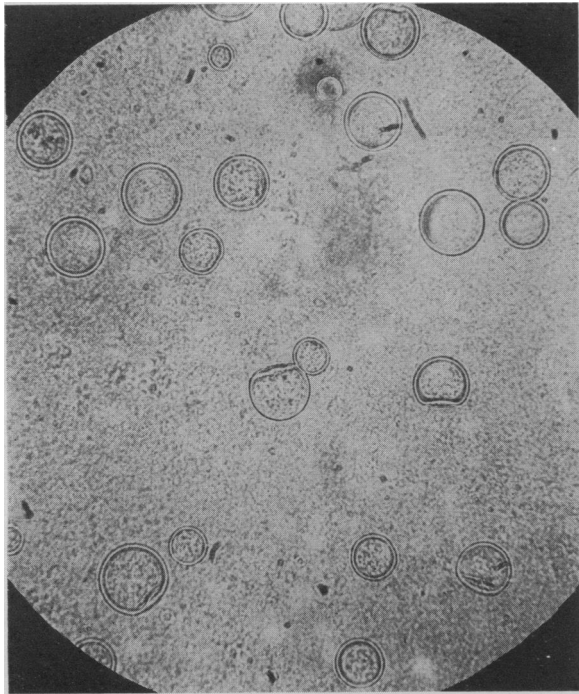


Fig. 1.—Effect of NaOH (10 per cent) in clearing pus preparation from experimental coccidioidal granuloma in guinea pig. Example of pseudo budding near center of illustration; actually it is but a matter of propinquity, referable to association of the two as originating in a common "ascus."

rations is not practical. Cultures have to be depended upon.

Staining Methods.—These have limited place, certainly as compared to bacteriology, because fixation by heat is calculated to distort a larger mass of material such as obtains in many fungus structures. In the event that it is felt that finer details of structure must be realized, such as cannot be secured short of staining technique, fixation should be performed by "wet" techniques such as are used in protozoology. To be sure, some sort of results may be secured by ordinary bacteriologic methods or indeed sometimes good results will more or less fortuitously occur, but to secure optimum and consistent results day in and day out it is felt that the foregoing modes of procedure will be of advantage.

Staining "In the Wet."—To avoid distortions incident to dry fixation (particularly with large yeast cells) most materials may be stirred into a small drop of undiluted Giemsa stain¹² and examined directly under the coverslip in this medium. The glycerin content effects a certain clarifying influence, but of course lacks the softening qualities of NaOH which are so desirable when examining tough materials like keratin. After about fifteen minutes, staining will have developed; accelerated by exerting pressure on the coverslip, as by tapping. Thereafter, grades of differentiation may be induced at the discretion of the observer by flushing distilled water from one side of the coverslip to the other.

India Ink Technique.¹³—This, performed in the wet, is a modification of the well-known Burri technique. Its justification is in demonstrating otherwise invisible or difficult to stain accumulations around (particularly yeast) cells such as are present in gloea formation. Yeast cells in fluid medium are the formations particularly susceptible to this technique, although other circumstances might arise for its employment. It is restful to the eye in prolonged observations.

Permanent Staining.—None of the preceding are suitable for permanent staining. As a matter of fact, whereas a wide range of dyes will stain the internal structures of the fungus cell, there are none which satisfactorily bring out the fungicellulose wall externally. Thus far I have found osmic acid the most serviceable; it stains fungicellulose faint brown. Of course, a counterstain may be superadded if it is desired to bring out internal structures yet farther. We critically need a *specific* stain for fungicellulose; from the fact that its composition is prone to vary *in vivo* with its environment, it is quite possible that by preliminary treatment *in vivo* with substances such as iron, such a specific stain might be developed.

Sabouraud's Medium.—This is a thing of the past—at least as originally postulated. Professor Sabouraud has personally communicated to me that he no longer uses crude maltose. The originally recommended Chanut brand is no longer on the market; indeed, Sabouraud has recently recommended honey as a substitute.¹⁴ From work in my laboratory,¹⁵ I feel that crude American glucose is an efficient substitute. Particular pains must be taken, however, as to the brand of peptone employed. The originally recommended

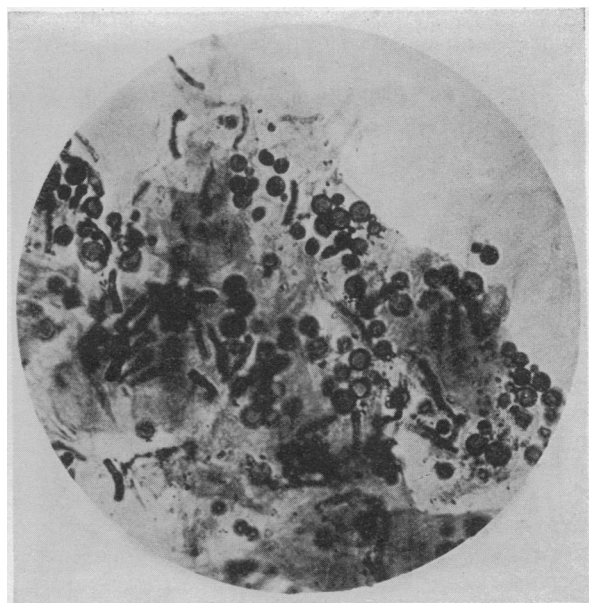


Fig. 2.—Scrapings from tinea versicolor to illustrate possibilities of staining "in the wet" by Giemsa. Both *Microsporon furfur* and bacteria are brought out in this material.

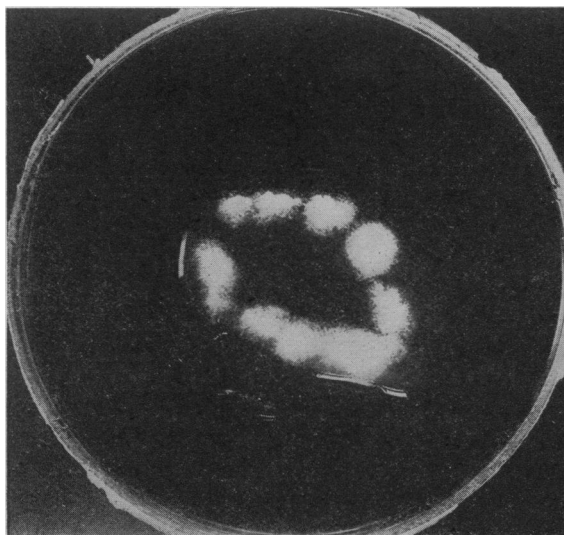


Fig. 3.—Employment of gentian violet medium in securing pure cultures.

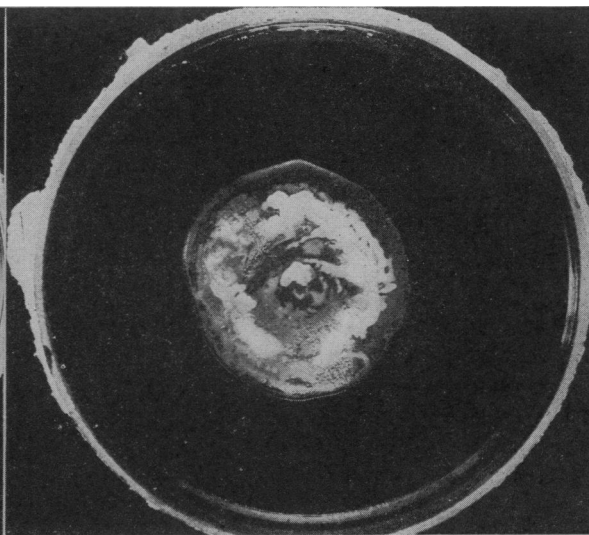


Fig. 4.—Same materials as in Fig. 3, but without gentian violet.

Chassaing is still available* and should be adhered to where it is proposed to faithfully elicit the standard gross cultural characteristics.

Fungi, considered by and large, develop well at such a low P^H as the 4.6 of Sabouraud's medium, besides at the $7.1 < >$ of bacteriologic media. This low P^H is automatically taken care of by the employment of Chassaing's peptone. It is of course particularly well known that carbohydrate (glucose) promotes luxuriance of growth in this class of plants. The advantage of luxuriance (but only provided it takes place under perfectly standard conditions) lies in the fact that thereby a particular conformation will be induced in the colonies, the different configurations being contingent upon the different permutations and combinations of factors like luxuriance, texture, moistness, production of conidia (powderiness), coarseness of hyphae, and so forth. Glucose is also calculated to bring out a pink (or other) color in colonies which otherwise may be latent.¹⁶

It should be distinctly understood that the rank and file of fungi will also grow on standard bacteriologic media and that the function of the glucose in mould mediums is in the direction of inducing gross characteristics. Indeed, glucose-free medium has some advantages (prevention of pleomorphism) for carrying along cultures in the laboratory.

Securing Pure Culture.—The low P^H previously mentioned eliminates a large number of contaminating bacteria; however, additional help-meets may be secured in special cases where it is found that this does not suffice. Inasmuch as both the hyphomycetes and the yeasts withstand gentian violet in concentrations of from 1:100,000 up to 1:2000, this agent may be employed as a restrainer, at least for the Gram-positive bacteria. A preliminary "drying out" of materials like pus over a period of two to three days does not vitiate

some fungi, but will destroy many bacteria. This experience has been known to be very valuable in isolating blastomyces and sporotrichum. It is to advantage to crush coarser particles of fungus substances like the grains of Madura foot and actinomyces before planting.

Repeated examinations are often necessary before finally securing a positive result. Only by a realization of this peculiarity, or better by experience, will the laboratory man bring the full complement of mycotic cases to light. This holds good not alone in superficial dermatoses but also in blastomycosis and sporotrichosis. Positive cultures, however, fortunately for you, are rather regularly obtained in coccidioidal cases.

Direct Examination of Culture Tubes on the Microscope Stage.—Advantage should be taken of the grossness of fungus structures by resort-

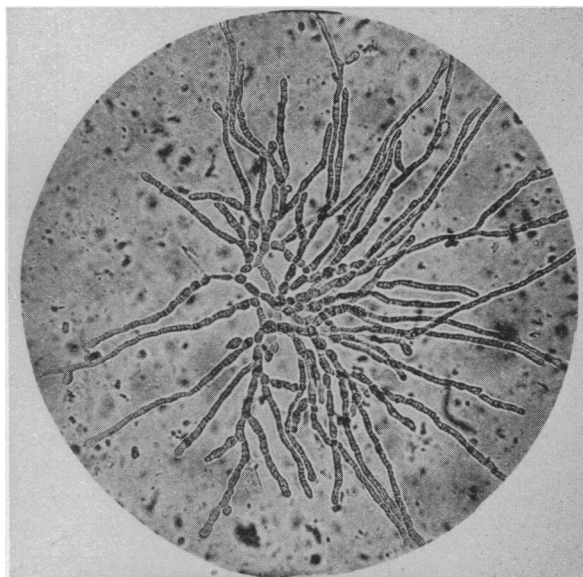


Fig. 5.—Hanging drop preparation of *Sporotrichum councilmanii* about five years old.

*Dolbey and Company, 3627 Woodland Avenue, Philadelphia, Pennsylvania.

ing to this method. By selecting thinner positions, like the uppermost, drier portions of the medium, and at its margins, the finer details of architecture may be readily determined as the fungus extends more or less out onto the walls of the tube. Of course only such information as will be secured by low powers of the microscope is avail-

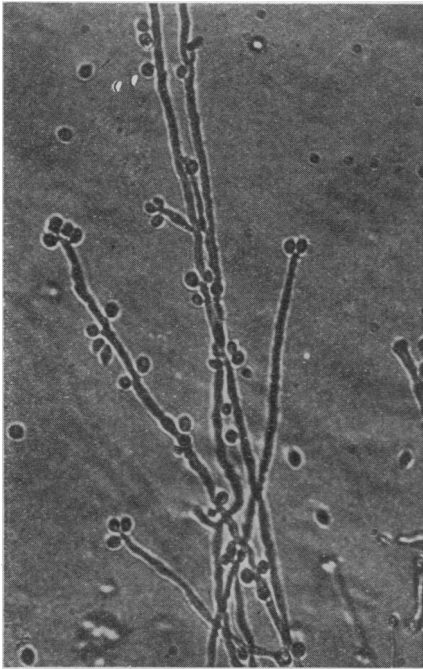


Fig. 6.—Illustrating how delicate the attachments of conidia are to hyphae in *Sporotrichum schenckii*. Since the grouping and arrangement of such conidia are essential to determination of the genus, the superiority of hanging drops over standard bacteriologic smears is obvious.

able in this way. Advantage may be taken of this maneuver to detect the earlier stages of growth of sporotrichum, planting material immediately at the edge of the substrate. Under these circumstances, one can recognize germinating fungus cells, when present, within from twenty-four to forty-eight hours. This does not, of course, suffice to determine the species, but indicates, when present, that fungi are present in the materials, and this information taken into consideration with the clinical circumstances may be sufficient to justify prompt embarkation upon anti-fungus treatment.

Hanging Drop.—Due to the complex architecture of fungus colonies as compared to bacteria, information is gained in the hanging drop as it is not for the bacteria. The particular grouping of spores along hyphae, spiral formations of hyphae, modes and frequency of branching, and so forth, are not distinguishable in ordinary smear preparations because the formations are likely to become disarranged. Due to the bulkiness of the fungus colonies and the necessity for a certain amount of oxygen the chamber of van Tieghem is the choice. In using it, sufficient time should be allowed for the full development of all the

differential "organes" that may be expected to develop—on the average, three weeks. It is difficult to secure *permanent* preparations of the latter without disarranging the more or less delicate attachments and arrangements of spores, etc. In my experience, the closest maintenance is secured by fixing in *vapors* of osmic acid, succeeded by hardening in *vapors* of alcohol. After this, the preparation may be allowed to evaporate just short of dryness, and mounted in Farrant's medium.

Clearing Agents.—Inasmuch as some of the fungi, as for instance those in Madura foot, are deeply pigmented, certain details may be difficult to make out microscopically. Sometimes potassium hydroxid will correct—is sufficient for clearing; if not, a proprietary, diaphonal,* at once bleaches and clears the preparation in a remarkable way. It acts too energetically (overclears) for routine fungus work, but for extremely thick material and deeply pigmented ones it is quite serviceable.

Iodids and Bromids in the Urine.—Inasmuch as these halogens may induce fungating, ulcerating granulomas of the skin, the pathologist should be prepared to bring the urinary data into service. The starch test for the former I scarcely need mention; for bromin, the Wile-Belote¹⁷ technique, employing fluorescin paper, should be taken advantage of. To five cubic centimeters of urine add a few crystals of potassium permanganate followed by one cubic centimeter of concentrated sulphuric acid. Test fumes of free bromin by filter paper strips dipped in fluorescin solution (change from faint yellow to pink).

Torula in Spinal Fluid.—In view of the occasional rôle played by this class of microorganism, the fact that they have been mistaken at first for red blood cells¹⁸ and the fact that the possibili-

* Leitz, Inc. New York City.

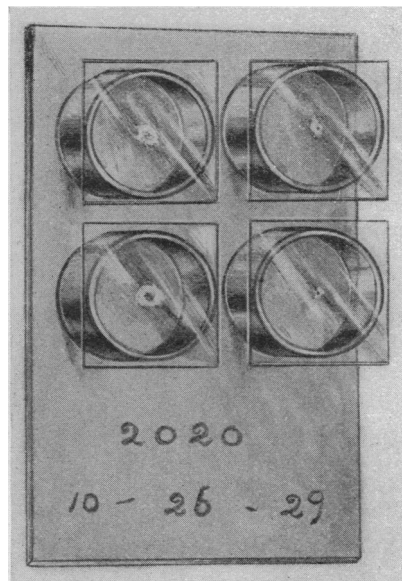


Fig. 7.—The van Tieghem cell. Units united and hermetically sealed with vaselin. Plantings are routinely made in quadruplicate in this laboratory.

ties of cerebrospinal treatment with iodid has not been exhausted, the routine inquiry for yeast in (selected cases of) clinically tuberculous meningitis appears advisable. This is all the more so in view of the absolutely black prognosis in this form of meningitis.

Leishman-Donovan Bodies.—As close as California is to Mexico, and with her flourishing coastwise commerce, I take it that routine preparations for Leishman-Donovan bodies should be made at least until the California profession has become thoroughly oriented as to the status of leishmaniasis here. With the Brazilians,¹⁹ leishmaniasis is an outstanding differential diagnostic problem in connection with blastomycosis and coccidioidal granuloma.

Actinomycosis.—I have learned by bitter experience that other microorganisms than actinomycosis may produce radiating and more or less concentrically zoned colonies of large size which closely mimic ray fungi. Davis²⁰ has rendered us a service in calling attention to these actinomyces-like granules in the tonsil. They are in no way related to true actinomyces and consist of bacilli, streptococci, and spirilla. They are not pathogenic. On the other hand, it will be recalled that true actinomyces are to be found in the tonsils of the hog.²¹

The actinobacillus²² of the veterinarian should be recalled when inquiring acutely into actinomyceslike granules. In every case the granules should be stained in tissue by Gram to determine whether there are streptothrix filaments in its interior; such must be invariably present in true actinomycosis. The roentgenologist will not be surprised when I remind that Sanford (loc cit) was not able to find a case on record at the Mayo Clinic (which is in an actinomycosis hotbed) where the bone was involved; this is in striking contrast to the lower animals, where osseous hyperplasia is such a conspicuous feature.

Madura Foot.—I only mention this to bring up the subject of the taxonomic determination of the rare and unusual species, genera, or even families of fungi which are calculated to be met in that affection. I have just determined, for instance, what appears to be *Scedosporium sclerotiale* in a case originating in Alabama.²³ Determinations of this sort, which are off the beaten path, are tremendous strains upon the time of the pathologist. The plant pathologist, on the other hand, is thoroughly familiar with such higher forms, and in a comprehensive plan for mycological centers such an individual should find place as a consultant on the staff. He is calculated not alone to help the pathologist play fair with himself, but to bring much in the way of technique and the most recent developments in fungous biology to the service of medical mycology.

Miscellaneous Fungi.—This paragraph finds place only to round out the list of deep mycoses. Apart from actinomyces, coccidioides, and so forth, already discussed, there are dozens of other

species which have been reported as more or less isolated examples of reputed pathogens (mucor, penicillium, cephalothecium, glenosporea, hormodendrum, hemispora, acladium, acremoniella, entantiothamnus, etc.). They have occurred in ulcers or "gummo ulcers." Naturally, insufficient data have accumulated to permit any generalizations being made; the concern of the pathologist at present is in identifying the species and recording it as fully as possible for future use in collecting and analyzing such cases.

Animal Inoculation.—Discretion must be exercised in selecting the laboratory animal. For sporotrichosis the rat is of choice, for blastomycosis the mouse and rat, while for coccidioidal granuloma the guinea pig is quite susceptible. In view of the extreme insusceptibility of rabbits to sporotrichosis and blastomycosis, it is probable that for each fungus a standard curve of pathogenicity could be constructed as reckoned upon the different laboratory animals. This would be of service in evaluating the virulence of different strains of coccidioides, etc., and I believe should be among the earlier items to be established in research on coccidioidal granuloma. The dog has not been sufficiently employed, and should not be omitted when anything like comprehensiveness is aimed at. It has been found most useful in immunologic work on aspergillosis.²⁴

Immunology.—Advances have not taken place in this field to the extent that they have in the bacteriological because bacteriology ought to, always has, and always will overshadow mycology. An equally weighty reason has been a notorious lack of specificity, most striking in the skin tests but also obtaining in the complement fixation ones. The agglutination tests for sporotrichosis, indeed, have until very recently stood rather alone in diagnostic value. At all of the others severe and well-deserved criticism has been leveled.²⁴ The probability is that the antigens have not been sufficiently fractionated and that what fractions are obtained are group ones largely referable to fungicellulose more or less *per se*. It is notoriously discomfiting that the tuberculin test is positive in even some of the superficial mycoses, to say nothing of the deeper ones, and vice versa. In re coccidioidal granuloma, the recent publications of Jacobson²⁵ and of de Fonseca²⁶ are distinctly encouraging; all that is needed is confirmation of their results.

Mycologic Centers.—I have already hinted at the very special position which mycology occupies in medical biology. Perhaps in the preceding paragraphs this has been exemplified. After this it goes without saying that the Pacific Coast is beyond question one of the regions which should be represented by a mycologic center; geographically, economically, and now opportunistically, it deserves this advantage. A stock culture collection of fungi is maintained, it is true, in Chicago,*

*American Type Culture Collection, John McCormick Institute for Infectious Diseases, 637 South Wood Street, Chicago, Illinois.

but that is only a part of a large whole. As stated elsewhere, it is too much to expect that pathologists become thoroughly versed in the intricacies of mycology; indeed, it is quite unnecessary. Such a center should have, however, at least one mycologic expert as departmental head, with one or two assistants and appropriate technical personnel as a beginning. To finance such a center I have only to cite the "Golden State"; the ability and materials which you have here are unsurpassed anywhere in the United States.

As we conclude, and look backward over the path we have traveled this morning, let us do it California-wise. Perhaps there are three eminences that dominate the landscape: first, the grumbling volcano "coccidioidal granuloma"; secondly, the butte "clinical"; and thirdly, the potentially commanding peak of all—laboratory examination." Equip the last, now, as a mycologic center and thereupon with the assistance of "butte clinical" you become dynamically commanding. Advances you are bound to make against coccidioidal granuloma and the other deep mycoses, if by no more than sheer effort, with your present forces; they will work much more efficiently, however, with the reinforcements added from a mycologic center.

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PULMONARY HEMORRHAGE*

AN INVESTIGATION OF THE EXCITING FACTORS IN TUBERCULOUS HEMOPTYSIS

Clinical Prize Paper of the Fifty-Ninth Annual
Session of the California Medical Association

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INTRODUCTION

PULMONARY hemorrhage is one of the most fearful and most frequent of the complications of pulmonary tuberculosis. According to Osler more than half of the patients with this disease suffer from some degree of pulmonary hemorrhage during the course of their illness. Since tuberculosis is responsible for nearly one-tenth of the average annual death rate, every twentieth person in this country suffers from hemoptysis from this cause at least once during his lifetime. It may appear as the first symptom in a person in whom the existence of tuberculous infection has never even been suspected, or it may occur as the last fatal event in a long-drawn-out struggle with chronic fibroid phthisis. Nevertheless, in spite of valuable work that has been done in the clinical, pathological, and experimental investigation of this phenomenon, pulmonary hemorrhage remains today nearly the mystery to the physician that it presents to the layman.

Pulmonary hemorrhage usually appears in the course of an advancing tuberculous process in the lungs, but why it appears when it does and under the conditions that usually prevail at that time is little understood. It is not a necessary consequence of the destructive lesion, since the tubercu-

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